IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

IN RE: MARSHALL, WILLIAM E.	)
	) **APPEAL NO. _____**
SERIAL NO: 09/883,550	)
	)
FOR: METHODS AND COMPOSITIONS	)
FOR MODULATING IMMUNE SYSTEMS	) **BRIEF ON APPEAL**
OF ANIMALS	)
	)
FILED: JUNE 18, 2001	)
	)
	)
GROUP ART UNIT: 1638	)

To Commissioner for Patents
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I. REAL PARTY IN INTEREST

The real party of interest in the present appeal is William E. Marshall of Naples, Florida.

II. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences regarding the present appeal of U.S. Application No. 09/883,550 which would be relevant to the Board's decision in this appeal.

III. STATUS OF CLAIMS

Claims 1-22 were originally submitted June 18, 2001. In a Restriction Election dated October 10, 2002 Appellant elected Group 1, claims 1-19. In an Amendment dated March 17, 2003 Appellant amended claims 1, 4-8, 10-13, 15 and 17 and canceled claim 9. In an Amendment After Final dated September 29, 2003 Appellant amended claims 1, 12 and 17 and canceled claims 2-3. In a *Substitute* Amendment After Final dated October 7, 2003 Appellant again amended claims 1, 12 and 17 and canceled claims 2-3. A Notice of Appeal was filed November 20, 2003. An Advisory Action was issued on December 22, 2003 not entering Appellant's amendments. A Request for Continued Examination (RCE) including a Preliminary Amendment and §132 Declaration of Dr. Marshall was filed January 19, 2004. In an Amendment filed June 30, 2004 Appellant amended claims 1, 4 and 18. In an Amendment After Final dated December 20, 2004 Appellant amended claim 1. An Advisory Action Before the Filing of an Appeal Brief was issued on February 10, 2005 entering Appellant's amendments. A Request for Continued Examination (RCE) was filed April 18, 2005. A telephonic conference was held on May 25, 2005 with Examiner

Robert A. Zeman, William E. Marshall (Inventor) and Lila A.T. Akrad (Applicant's Representative) where no agreement with respect to the claims was met. In an Amendment filed October 14, 2005 Appellant amended claims 1 and 13. In an Amendment After Final filed April 24, 2006 Appellant amended claim 1 and canceled claim 13. An Advisory Action Before the Filing of an Appeal brief was issued May 19, 2006 entering Appellant's amendments. A Notice of Appeal was filed May 23, 2006. This is an appeal of the Final Rejection dated February 23, 2006 and of the Advisory Action dated May 19, 2006, finally rejecting claims 1, 4-8, 10-12, and 14-19. The claims here appealed are claims 1, 4-8, 10-12, and 14-19.

IV. STATUS OF AMENDMENTS

A Restriction Election was filed October 10, 2002. An Amendment was filed March 17, 2003. An Amendment After Final was filed September 29, 2003. A *Substitute* Amendment After Final was filed October 7, 2003. A Notice of Appeal was filed November 20, 2003. A Request for Continued Examination (RCE) including a Preliminary Amendment and §132 Declaration was filed January 19, 2004. An Amendment was filed June 30, 2004. An Amendment After Final was filed December 20, 2004. A Request for Continued Examination (RCE) was filed April 18, 2005. An Amendment was filed October 14, 2005. An Amendment After Final was filed April 24, 2006. A Notice of Appeal was filed May 23, 2006.

V. SUMMARY OF CLAIMED SUBJECT MATTER

This invention relates to methods and compositions for modulating immune responses of animals or humans. More particularly, the invention relates to methods of modulating immune responses of animals or humans by administering effective amounts of a partially purified composition prepared from a mixture released by the stressing of bacteria after they have been grown under specified conditions. This composition includes stress response factors that activate and modulate circulating macrophages. (*See* Original specification as filed, p. 1).

The present inventors have found that the stress-response-factors (SRFs), between 0.5 and 10. kDa are a rich new source of natural, normally-occurring, co-evolutionarily evolved immune modulators that can be safely used to protect animals and humans from infections and over-stimulation of their immune system. This fraction was shown in cell cultures to induce the release of cytokines, IL-1, IL-6, and TNFα and decrease the expression of individual surface receptors CD-14 and CD-16 on macrophages, thereby re-centering a dysfunctional immune system and desensitizing it to a subsequent lethal challenge of injected endotoxin, LPS. (*See* Original specification as filed, pp. 19-21). Furthermore, in vitro testing indicates their potential role as adjuvants by stimulating the release of IL-12. (*See* Original specification as filed, p. 5).

An additional discovery is the finding that feral colonies of bacteria yield more oligomeric SRFs than non-feral or laboratory strains. (*See* Original specification as filed, p. 5). However, after stressing, laboratory strains assume the more robust growth characteristics of feral strains and the subsequent release of more SRFs when stressed. (*See* Original specification as filed, p. 5). The release of SRFs can be tracked by

measuring their peak of absorption at 254 nm that typically is associated with nucleotides. (*See* Original specification as filed, pp. 16-17).

The discovery of the release of immune-activating and modulating factors has broad implications to improving the immune response through diets and pharmaceutical preparations for humans and animals. Products, e.g. milk, cheese, yogurts contain viable bacteria, which, when transferred to the nutrient deprived and pH neutral environment of the mouth release SRFs. If such products were formulated to extend the dwell-time in the mouth, more SRFs would be released, activating and modulating a greater local immune response. (*See* Original specification as filed, p. 5). Material relevant to the appealed claims is described throughout the Specification.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Claims 1, 4-8, 10-12, 14-15 and 17-19 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over De Vuyst et al. (Microbiology, Vol. 142, 1996, pp. 817-827).

B. Claims 1, 4-8, 10-15 and 17-19 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over De Vuyst et al., cited in A., in view of Nanji (U.S. Patent No. 5,413,785 - IDS-2).

C. Claim 16 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over De Vuyst et al., cited in A., in view of Perdigon et al. (Journal of Food Protection Vol. 53, No. 5, pp. 404-410, 1996 - IDS-2).

VII. ARGUMENT

A. The Examiner's Rejection of Claims 1, 4-8, 10-12, and 14-19 Under 35 U.S.C. § 103(a) Has Been Improperly Maintained

The Examiner rejects claims 1, 4-8, 10-12, and 14-19 as unpatentable as the "subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains". *See* 35 U.S.C. § 103(a). The Examiner states that claims 1, 4-8, 10-12, 14-15 and 17-19 are obvious where "De Vuyst et al. suggests that low molecular weight proteins (bacteriocins) are produced by stressed bacteria" (*See* December 17, 2002, Office Action, p. 7). The Examiner further states "the bacteriocins disclosed by De Vuyst et al. are not limited to the aggregate form." In fact, De Vuyst et al. disclose that to harvest maximum bacteriocin levels it is advisable to use conditions that prevent aggregations (see p. 824, right hand column). Consequently, De Vuyst et al. disclose bacteriocins comprising the 6 kDa monomers that meet the limitations of the instant claims." (*See* May 9, 2006 Advisory Action, p. 3).

The Examiner further states that claims 1, 4-8, 10-15 and 17-19 are obvious where "De Vuyst et al., in view of Nanji as disclose the administration of lactic acid bacteria to animals for protection against endotoxin-mediated shock" (*See* December 17, 2002, Office Action, p. 8). The Examiner then states that claim 16 is obvious where "De Vuyst et al., in view of Perdigon et al. discloses the use of low molecular weight proteins as adjuvants" (*See* December 17, 2002, Office Action, p. 9).

The Examiner's rejections are improper. In order for the cited references to be construed as prior art which would have been obvious to a person having ordinary skill in

the art to which the subject matter pertains all of the claim elements must be taught or suggested by the combined references. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991) and MPEP § 2142. Applicant believes that the Examiner's rejections are improper and that the cited references do not teach, nor suggest, the claimed unique invention and that the non-obviousness requirement has been satisfied for several different reasons, described in detail *infra.*

1. The Law of Obviousness

When applying 35 U.S.C. § 103, the following tenets of patent law must be adhered to: (a) the claimed invention must be considered as a whole; (b) the references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination; (c) the references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and (d) reasonable expectation of success is the standard with which obviousness is determined. *See Hodosh v. Block Drug Co., Inc.*, 786 F.2d 1136, 1143 n.5, 229 USPQ 182, 187 n.5 (Fed. Cir. 1986) and MPEP § 2141. To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest *all* the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must *both* be found in the prior art, not in applicant's disclosure. *See In re Vaeck*, 947 F.2d 488, 20

USPQ2d 1438 (Fed. Cir. 1991) (emphasis added) and MPEP §§ 2142 and 2143. Applicant submits that the cited references do not teach, nor suggest, all the elements of the claimed unique invention.

In determining the differences between the prior art and the claims, the question under 35 U.S.C. § 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious. *See Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 USPQ 871 (Fed. Cir. 1983); *Schenck v. Nortron Corp.*, 713 F.2d 782, 218 USPQ 698 (Fed. Cir. 1983) and MPEP § 2141.02. A prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention. *See W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984) and MPEP §2141.02. Applicant asserts the references cited by the Examiner do not teach or suggest every element of the claimed invention, which must be identically disclosed, in a single reference. *See Corning Glass Works v. Sumitomo Electric*, 9 USPQ2d 1962, 1965 (Fed. Cir. 1989). Furthermore, when the motivation to combine the teachings of the references is not immediately apparent, it is the duty of the examiner to explain why the combination of the teachings is proper. *See Ex parte Skinner*, 2 USPQ2d 1788 (Bd. Pat. App. & Inter. 1986) and MPEP § 2142.

It is also important to note that when prior art references require selective combination to render obvious a subsequent invention, there must be some reason for the combination other than hindsight obtained from the invention itself. It is critical to understand the particular results the new method achieves. *See Interconnect Planning Corp. v. Feil*, 774 F.2d 1132 (Fed. Cir. 1985). The mere fact that references can be

combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *See In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990) and MPEP § 2143.01. Therefore, it is respectfully submitted that any rejection based on the cited references is overly broad because the combination of the references does not render the Applicant's invention, as defined in the claims, obvious. None of the cited references alone or in combination teaches, suggests or even mentions stress response factors described in Applicant's specification, which are distinct from bacteriocins. In addition, SRFs do not kill bacteria as do the cited bacteriocins. Applicant strongly asserts that neither the suggestion of all the elements of the claimed unique invention of the present application nor the expectation of success is taught for one ordinarily skilled in the art in the references cited by the Examiner.

2. The Examiner's Conclusion of Obviousness is Based on an Incorrect Application of the Law to the Claimed Invention

a. Claims 1, 4-8, 10-12, 14-15 and 17-19 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over De Vuyst et al. (Microbiology, Vol. 142, 1996, pp. 817-827)

Applicant respectfully submits that the Examiner did not make out a *prima facie* case of obviousness as the cited reference fails to teach or suggest all of the elements of Applicant's claimed invention. MPEP § 2142. Although DeVuyst et al. and Applicant both use the seemingly similar phrases of "stress factors" (De Vuyst et al., p. 818) and "stress response factors" (Applicant, original specification as filed, p. 1), the phrases clearly have different meanings when read in context. De Vuyst et al. use the phrase "stress factors" to refer to unfavorable *stressed* growth conditions for bacteria. (*See* De

Vuyst et al., p. 818, right column, stating, "Thus, manipulation of the cell environment can stimulate bacteriocin production. The latter can be induced by unfavourable growth conditions, so-called stress factors"). In contrast, Applicant uses the phrase "stress response factors" merely as an epithet to describe a newly discovered composition produced by growing bacteria under *normal* growth conditions and subsequently *releasing* the claimed composition by exposing the bacteria to stressors after normal growth. It is vital to note that bacteria are grown under stressed conditions in De Vuyst et al. to produce bacteriocins whereas in stark contrast bacteria are grown under normal conditions in the present invention and then removed from their growth environment wherein they release "stress response factors".

Furthermore, De Vuyst et al. teach the production of a bacteriocin produced by *Lactobacillus amylovorus* DCE 471 which is bacteriocidal towards closely related *Lactobacillus* strains (p. 818). De Vuyst et al. further disclose that the bacteriocin-producing lactic acid bacteria could potentially be added to foods to stimulate bacteriocin production (p. 825). It is submitted that the Examiner is misinterpreting Applicant's invention, which is not related to bacteriocins or other compositions with bactericidal properties. *See* § 132 Declaration of inventor Dr. Marshall, which clearly establishes that the SRF compositions of the invention do not include bacteriocins or other compositions with bactericidal properties (Exhibit 1). The declaration details several experiments conducted using the standard methods in the art and even used by De Vuyst exposing the test strain for bacteriocins, *Lactobacillus helveticus*, ATCC 15009, to the SRF compositions. The results show that the SRF compositions do not exhibit bactericidal activity. The results as depicted in Figure 1 demonstrate that the preparations of the

invention obtained from *L. monocytogenes, L. plantarum,* and *E. faecium* do not inhibit growth of *Lactobacillus helveticus*. This is in stark contrast to the bacteriocin Nisin, which is shown at the asterisk. Figure 2 shows that stressing *L. monocytogenes*, or even twice stressing *L. plantarum* and *E. faecium* or stressing heat killed *L. plantarum* and *E. faecium* do not result in bacteriocidal activity against *L. helveticus*. Finally in Figure 3, 7 test strains (5 of *L. plantarum* and 2 of *E. faecium*) were used both as SRF generating strains and as test strains. Again, the bacteriocin Nisin inhibited all 7 strains while the SRFs collected from the same strains as well as from *L. caseii* did not inhibit growth. Further support that Applicant's SRFs are not bacteriocins is demonstrated by the specific wavelengths at which absorbance is measured for the bacteriocin and SRF compositions. The concentration of bacteriocins is measured at a wavelength appropriate for detecting proteins, 220 or 280 nanometers (nm). *See* General Biochemistry, Fruton & Simmonds, Wiley & Sons, 1955, submitted in a Supplemental Information Disclosure Statement, April 18, 2005. In contrast, Applicant's teach determining the presence of SRFs by measuring their absorbance at a wavelength appropriate for detecting nucleotides, specifically at 254 nm, not 220 or 280 nm. (*See* Original specification as filed, at p. 16-17) Moreover, the bacteriocin Nisin does not show peaks of absorption at wavelengths other than 220 and 280, which is to be expected, since it does not contain adenine, cytosine, guanine, thymine or uracil -- the bases responsible for absorbing at wavelengths around 260 nm and found in nucleic acids. However bacteriocin does contain several aromatic amino acids that absorb UV in the 270 - 280 nm range which explains why its concentration was estimated by its coefficient of extinction at 280 nm. *See* General Biochemistry, Fruton & Simmonds,

Wiley & Sons, 1955, submitted in a Supplemental Information Disclosure Statement, April 18, 2005.

Furthermore, the De Vuyst et al. reference does not suggest in any way that bacteria could or should be sequentially stressed chemically, physically, or biologically for at least two or more sequential periods of stress of twenty minutes or less to release a stress response product, let alone that the stress response product released by the bacteria using this method would produce more potent stress response factors than those produced by exposing bacteria to longer periods of stress as taught by Applicant. (*See* Original specification as filed, at p. 12, lines 10-18; Table 9 of original specification as filed, at pp. 27-28.)

While the De Vuyst et al. reference discloses a general idea for increasing production of bacteriocins by manipulating environmental conditions during growth, the reference does not disclose or suggest that sequentially stressing bacteria after the completion of growth will produce a stress response product. De Vuyst et al. state that "[a]pparently, bacteriocin production is stimulated by less favorable growth conditions, so called stress factors, such as low temperatures, low specific growth rates, potentially toxic compounds such as sodium chloride, ethanol and oxygen". De Vuyst et al., at p. 825, left column. At most, this statement is an invitation to experiment. One skilled in the art reading De Vuyst et al. would not have recognized or appreciated the reference as teaching the importance of exposing bacteria sequentially to stress.

In fact, the De Vuyst et al. reference teaches away from the present invention because "a person of ordinary skill, upon reading the reference, ... would be led in a direction divergent from the path the Applicant took." *In re Gurley*, 27 F.3d 551, 31

USPQ 2d 1130, 1131 (Fed. Cir. 1994). For example, Applicant describes exposing bacteria, after growth, to biological, chemical or physical stress for at least two or more sequential periods of stress of approximately twenty minutes or less so that the bacteria release a stress response product comprising stress response factors (SRFs). De Vuyst et al. describe exposing bacteria to less favorable growth conditions concurrently. De Vuyst at p. 819, left column, first paragraph under results section (growing bacteria at a temperature of 30° C at a controlled pH of 5.0). Thus, the De Vuyst et al. reference teaches away from the claimed invention because an ordinary skilled artisan upon reading the De Vuyst et al. reference would be led in a different direction than Applicant – exposing bacteria to stress conditions concurrently during growth to stimulate bacteriocin synthesis de novo rather than sequentially after growth to force the release of SRFs.

Nowhere does the De Vuyst et al. reference state that a particular length of exposure time would be any more advantageous than another in producing a stress response product, much less exposing bacteria to stress conditions sequentially for twenty minutes or less. There is no teaching, suggestion or motivation from the De Vuyst et al. reference to modify the length of exposure time of bacteria to stress. "Even when obviousness is based on a single prior art reference, there must be a showing of a suggestion or motivation to modify the teachings of the reference. *See B.F. Goodrich Co. v. Aircraft Braking Sys. Corp.*, 72 F.3d 1577, 1582, 37 USPQ2d 1314, 1318 (Fed. Cir. 1996)." *In re Kotzab*, 217 F.3d 1365, 1370, 55 USPQ2d 1313, 1316-17 (Fed. Cir. 2000). Moreover, De Vuyst et al. fail to appreciate or recognize that exposing bacteria to stress conditions sequentially for twenty minutes or less produces a stress response product of increased potency.

Thus, the De Vuyst et al. reference simply does not teach or suggest releasing a stress response product from bacteria by exposing the bacteria to biological, chemical or physical stress for at least two or more sequential periods of stress wherein each period of stress is defined by a period of stress exposure of approximately twenty minutes or less, filtering the product to remove substances having a molecular weight of greater than 10kDa to form a filtrate, and administering the filtrate to an animal for use in modulating the immune system of an animal. De Vuyst et al. does not teach each and every element of claim 1. The Office Action fails to meet its burden of establishing a *prima facie* case of obviousness. Thus, the present invention is not obvious. Claims 4-8, 10-12 and 14-19 dependent on independent claim 1 are likewise not obvious for the reasons argued above, plus the elements in the claims.

For the above reasons, a person skilled in the art would recognize that Applicant's claimed invention, claims 1, 4-8, 10-12 and 17-19, is not obvious over De Vuyst et al.

b. Claims 1, 4-8, 10-15 and 17-19 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over De Vuyst et al., in view of Nanji (U.S. Patent No. 5,413,785 - IDS-2)

Applicant asserts that even if the De Vuyst et al. reference and Nanji patent were combined, the combination would not have suggested the present invention to one skilled in the art because they do not teach all elements of the presently claimed methods. The U.S. Patent No. 5,413,785 (hereinafter referred to as '785) to Nanji teaches a unique bacterial strain, *Lactobacillus* GG, that is able to destroy Gram-negative bacteria by passing through the acid environment of the stomach, attaching to the lining of the intestine, and producing hydrogen peroxide and either bacteriocin or another anti-microbial substance. *See* Nanji, '785, Table 1, Col. 6, lines 28-39. These actions block the

attachment of bacteria and kill Gram-negative bacteria capable of releasing harmful endotoxin.

Neither the De Vuyst et al. reference nor the Nanji patent teaches or suggests that common harmless bacteria release immune-stimulating factors when subjected to stressors. Furthermore, they do not teach or suggest exposing the bacteria to biological, chemical or physical stress for at least two or more sequential periods of stress of approximately twenty minutes or less to release a stress response product that is less than 10 kDa from bacteria for use in modulating the immune system of an animal as found in the Applicant's claim 1. Thus, the prior art references, alone or combined, fail to teach or suggest all the claim limitations of the present invention. Therefore, claim 1 is not obvious. Claims 4-8, 10-12, 14-15 and 17-19 dependent on independent claim 1 are likewise not obvious for the reasons argued above, plus the elements in the claims.

For the above reasons, Applicant asserts that the combination of references cited by the Examiner do not teach or suggest the unique method of the present invention and thus would not be obvious to one of ordinary skill in the art. In fact, Applicant respectfully submits that any such suggestion would be merely hindsight application of the Applicant's specification and claimed invention to the cited references.

c. Claim 16 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over De Vuyst et al., in view of Perdigon et al. (Journal of Food Protection Vol. 53, No. 5, pp. 404-410, 1996 - IDS-2)

As discussed *supra*, the De Vuyst et al. reference does not teach exposing the bacteria to biological, chemical or physical stress for at least two or more sequential periods of stress of approximately twenty minutes or less to obtain the release of a stress

response product that is less than 10 kDa from bacteria for use in modulating the immune system of an animal as found in the Applicant's claim 1. Perdigon describes the administration of several species of *Lactobacillus* to mice to enhance antibody production to enteropathogens in the intestine. *See* Perdigon, p. 404. Nowhere does Perdigon mention or suggest stressing bacteria. Thus, Perdigon fails to supply the teachings that are lacking in De Vuyst et al. Clearly, the combination of references fail to teach or suggest the limitations of claim 1 and thus cannot render claim 1 obvious. Therefore, claim 16 depending from claim 1 is not obvious for the reasons argued above, plus the elements in the claims.

For the above reasons, Applicant asserts that the combination of references cited by the Examiner do not teach or suggest the unique method of the present invention and thus would not be obvious to one of ordinary skill in the art.

3. Conclusion as to Obviousness

When prior art references require selective combination to render obvious a subsequent invention, there must be some reason for the combination other than hindsight obtained from the invention itself. It is critical to understand the particular results the new method achieves. *See Interconnect Planning Corp. v. Feil*, 774 F.2d 1132 (Fed. Cir. 1985). Further, the cited references do not teach or suggest every element of the claimed invention, which must be identically disclosed, in a single reference. *See Corning Glass Works v. Sumitomo Electric*, 9 USPQ2d 1962, 1965 (Fed. Cir. 1989). Finally, the prior art reference (or references when combined) must teach or suggest *all* the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must *both* be found in the prior art, not in applicant's disclosure.

See In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991) (emphasis added) and MPEP §§ 2142 and 2143. Therefore, Applicant strongly asserts that neither the suggestion of the claimed unique invention of the present application nor the expectation of success is taught for one ordinarily skilled in the art in the references cited by the Examiner. Applicant is entitled to the scope of the invention as claimed.

In sum, Applicant has fully satisfied the legal standards for non-obviousness as set forth in case law and the MPEP. Appellant therefore respectfully requests that the Examiner's rejections under 35 U.S.C. § 103(a), be reversed.

For the above-stated reasons, it is submitted that the claims are in condition for allowance. The decision of the Examiner, therefore, should be reversed and the case allowed.

Enclosed herein please find the Appeal Brief and required fee of $250.00. Please also find enclosed an extension of time for two months, and the required fee of $225.00. If this amount is not correct, please consider this a request to debit or credit Deposit Account No. 26-0084 accordingly.

Respectfully submitted,

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Attorneys of Record

LATA/JCB

IX. EVIDENCE APPENDIX

Only evidence of record has been relied upon in this appeal.

Exhibit 1: 37 C.F.R. § 132 Declaration of inventor Dr. Marshall

First cited by Applicants in March 17, 2003 Amendment. Due to the figures being unreadable by the Examiner the § 132 Declaration was submitted in the Amendment After Final dated September 29, 2003 and again in the *Substitute* Amendment After Final filed October 7, 2003. The § 132 Declaration was then submitted in the Request for Continued Examination (RCE) dated January 19, 2004 in the Preliminary Amendment wherein the § 132 Declaration was entered by the Examiner in the non-final Office Action dated March 30, 2004.

X. RELATED PROCEEDINGS APPENDIX

There are no related proceedings in the present appeal of U.S. Application No. 09/883,550.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	**MARSHALL, William E.**
SERIAL NO:	09/883,550
FILED:	June 18, 2001
TITLE:	METHODS AND COMPOSITIONS FOR MODULATING IMMUNE SYSTEMS OF ANIMALS
GRP./A.U.:	1645
EXAMINER:	**ZEMAN, R.**
CONF. NO.:	1897
DOCKET NO:	P01936US5

132 DECLARATION OF WILLIAM E. MARSHALL

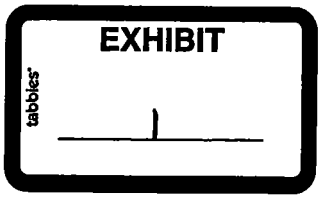
Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, William E. Marshall hereby declare the following.

1. I am the inventor on the above-identified case and am familiar with the prosecution including the Office Action dated May 30, 2003.

2. My background includes a Ph.D. in biochemistry from the University of Illinois, post-doctoral training at Uppsala University and Cambridge University, assistant professor of biochemistry at the University of Minnesota, director of technology development at General Foods Corp., president of the Microbial Genetics Division of Pioneer Hi-Bred International, member of the Iowa Academy of Sciences, chairman of the National Agricultural Research and Extension Users Advisory Board of the U.S. Congress, member of the advisory panel on biotechnology to the Office of Technology Assessment of the U.S. Congress, member of the advisory panel on intellectual property to the GATT, and associate professor of microbiology and immunology at the New York Medical College.



3. I understand that the Examiner has placed rejections on my application based upon one or more of the following references: De Vuyst et al., 1996 142 p. 817-827; Nanji, U.S. Patent No. 5,413,785; and Perdigon et al., 1990 J Food Production 53 (5) p. 404-410. This application details scientific argument and experimental evidence to refute the Examiner's contentions. The Examiner states that "De Vuyst et al. disclose methods of producing low molecular weight proteins from bacteria by subjecting them to a number of stresses. By definition, these proteins are stress response factors. De Vuyst et al. further disclose that these bacteriocins are able to kill or harm other bacterial species and suggest the use of said bacteriocins as food additives. Consequently it would have been obvious to one of ordinary skill in the art at the time the invention was made to have followed the suggestion of De Vuyst et al." Further, the Examiner states "Nanji discloses the administration of lactic acid bacteria to humans, livestock and other animals for protection against endotoxin-mediated shock. Therefore, it would have been obvious to one of ordinary skill in the art to use the bacteriocins by De Vuyst et al. in the treatment of methodologies of Nanji in order to take advantage of the immune enhancing effects of the bacteriocins while minimizing the complications associated with introducing a bacterial strain into the normal flora of an animal." Finally, the Examiner states "the teachings of De Vuyst et al. in view of Perdigon et al. disclose the use of lactic acid bacteria and the proteins produced therein as immunogens and adjuvants in the generation of protection from enteropathogens. It would have been obvious to one of ordinary skill in the art at the time the invention was made to use low molecular weight proteins disclosed by De Vuyst et al. as adjuvants for the induction of a immune response to another co-administered pathogen since Perdigon et al discusses the use of lactic acid bacteria (and the proteins produced by said bacteria) as adjuvants for enteropathogens (an increased immune response to said enteropathogens was also disclosed) and De Vuyst et al. disclose that proteins produced by lactic acid bacteria have an immunomodulatory effect." The present application is distinguishable from the cited references because De Vuyst et al, Nanji, and Perdigon et al. claim proteins. In contrast, I have determined that the SRFs are

oligoribonucleotides. Described herein are the methods and results used to identify the nature of the present invention's intracellular solutes.

4. Attached is Figure 4 from U.S. Patent No. 5,840,318 representing the separation of the intracellular solutes. There is no evidence of the presence of proteins or peptides among the claimed invention's intracellular solutes therefore, I assert the present invention's intracellular solutes are not bacteriocins (proteins/peptides) as suggested by the Examiner.

Figure 4 is a drawing of a Sephadex G-10 separation of the intracellular solutes (SRFs) typically released by the stressing of bacteria. It is the same Figure 4 in U.S. Patent No. 5,840,318, Methods and Compositions for Modulating Immune Systems of Animals, Marshall and Hoffmann. It shows that the <10kDa SRFs are a mixture of small components having maximum absorbance at 254nm, not 280 nm indicating that they are nucleotides, not proteins or peptides.

5. Also attached herewith and for the Examiner's consideration is a Xerox copy of a page from my lab notebook obtained from experiments conducted to refute several of the Examiner's contentions. The copy displays a figure (Figure 5) representing the separation of the intracellular solutes on HPLC (high pressure liquid chromatography) which indicate 13 peaks or components.

Figure 5 is a Xerox copy of a machine tracing showing the separation of the intracellular solutes (SRFs) on HPLC (high pressure liquid chromatography). The X-axis is the time of elution of the components in minutes; the Y-axis is the absorbance at 220nm. The effluents corresponding to the peaks was further examined by UV absorbance and showed maxima at 254 nm, typical of nucleotides and not 280 nm as one would expect from proteins. There is no evidence of the presence of proteins or peptides among the claimed invention's intracellular solutes therefore, I assert the present invention's intracellular solutes are not bacteriocins (proteins/peptides) as suggested by the Examiner. The tracing indicates 13 peaks or components in two groupings. Since the first group of nucleotides appearing as Peaks 3-6 were eluted immediately with an aqueous solvent, they are hydrophilic. The second group of peaks, labeled 19-23 are hydrophobic since they

were eluted with a solvent that was 50% methyl cyanide and 0.1% triflouro acetic acid. The claimed invention's intracellular solutes are a mixture of 10-13 major nucleotides, smaller than 10 kDa. Approximately half are hydrophobic and half are hydrophilic.

6. Attached herewith and for the Examiner's consideration is a Xerox copy of a page from my lab notebook obtained from experiments conducted to refute several of the Examiner's contentions.

Figure 6 is a photo and hand drawn table of a Thin Layer Chromatogram (TLC) of the SRFs less than 10 kDa released from *L. monocytogenes* and detected by UV light at 254nm. The large dark spot at the origin in Lane 4 represents Peak I from Sephadex. Staying at the origin of application indicates that is insoluble in the aqueous alcohol solvent and probably large, i.e., it corresponds to the hydrophobic group seen on the HPLC. The spots labeled A-D are components of Sephadex Peaks II-IV and correspond to smaller, hydrophilic nucleotides and the free base, uracil. The presence of uracil and the absence of thymine (TLC not shown) indicates that the nucleotides are from RNA, not DNA.

7. The Examiner states "[b]y definition, these proteins are stress response factors." Attached herewith and for the Examiner's consideration is a Xerox copy of a page from my lab notebook that presents a figure (Figure 7) that demonstrates my assertion that the present invention's SRFs are ribonucleotides, not proteins or peptides, smaller than 10kDa. This figure also represents my assertion that there is no evidence of the presence of proteins or peptides among the claimed invention's intracellular solutes as suggested by the Examiner.

Figure 7 is a photo and drawing demonstrating that SRFs less than 10 kDa from *L. caseii* are similar to those released by *L. monocytogenes*. It indicates that some oligoribonucleotides are hydrophobic and large while others are hydrophilic and small. It also shows that oligoribonucleotides are repeatedly released during exposures to pH neutral buffers. In Lanes 12 and 13 are the oligoribonucleotides from two washings of *L. caseii*.

8. Our invention is not limited to only stressing lactic acid bacteria. We have demonstrated that the phenomenon of stressed bacteria releasing immune-activating SRFs less than 10 kDa is also observed in Gram-negative bacteria, *S. typhimurium*, *E. coli*, and *K. pneumoniae*, a strict anaerobe, *B. coryneforme*, and the gram-positive pathogens, *S. aereus*, *L. monocytogenes*, and *S. pyogenes*. The use of harmless generally regarded as safe lactic acid bacteria has been emphasized only for purposes of minimizing manufacturing costs and allowing the technology to be practiced as a cottage industry. Further, the present invention is specifically related to how the bacteria use the aforementioned intracellular solutes to creat a dormant state and how the immune system has adapted an alert response to their sudden appearance. The claimed invention and the §132 Declarations submitted herewith and on March 17, 2003 show that the SRFs of the present invention are not proteins, polypeptides, or peptides and they do not contain amino acids but rather are oligoribonucleotides. Further, the SRFs of the invention do not alter growth patterns or the growth rates of other bacteria. Therefore, it is respectfully submitted the cited references do not teach or suggest the unique method of the claimed invention.

9. SRFs are a mixture of compounds having an absorption maximum at 254 nm, indicating the presence of nucleotides, not proteins. Proteins have a maximum at 280 nm.

10. The prior art of De Vuyst et al., Nanji and Perdigon as summarized by the Examiner teaches that proteins (bacteriocins) released by bacteria can kill other bacteria and, can also act as adjuvants. In contrast, the SRFs in my invention are not proteins, polypeptides or peptides; they do not contain amino acids but rather they are oligoribonucleotides. Further, the SRFs do not alter the growth patterns or the growth rates of other bacteria. I also assert that the earlier § 132 Declaration of March 17, 2003 showed that the released intracellular solutes of the claimed invention lacked any bacteriocin-like activity.

11. In addition, re-submitted herewith and for the Examiner's consideration are 3 photographs obtained from experiments conducted to refute several of the Examiner's

contentions. These photographs were submitted in the previous 132 Declaration of March 17, 2003 and noted by the Examiner as unreadable.

The protocols are detailed beneath the photos. The results show that neither total SRFs nor SRFs <10 kDa prepared from stressing 5 strains of *Lactobacillus plantarum*, 2 strains of *Enterococcus faecium*, *L. casei*, *L. acidophilus*, and *Listeria monocytogenes* contain bacteriocins. The release of SRFs was induced by transferring them from their growth media into Dulbecco's LPS-free phosphate-buffered-saline, pH 7.3 for 20 hours, at 37°C unless specified otherwise.

Figure 1 is a photo demonstrating that neither total SRFs nor those <10 kDa released by stressing bacteria results in the production of bacteriocins.

Figure 2 is a photo demonstrating that stressing higher levels of bacteria, or stressing bacteria twice or heat-killing bacteria does not result in the production of bacteriocins.

Figure 3 is a photo demonstrating that neither total SRFs nor those <10 kDa released by stressing bacteria results in the production of bacteriocins tested against a lawn of a mixture of lactic-acid bacteria.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Date: 9-25-2003

William E. Marshall

FIGURE 1

SRFs Do Not Possess Bacteriocin-like Activity

L. monocytogenes, L. plantarum and E. faecium do not release bacteriocins by exposure to pH neutral buffer or pH 4 buffer.

PROCEDURE:

A lawn of *Lactobacillus helveticus* ATCC 15009 was prepared by pour plating the test strain used by De Vuyst for the detection of bacteriocin.

Three hr later:

- 10 µl containing 25 µg of Nisin, a known bacteriocin from Sigma-Aldrich was added to the solidified agar at the top spot in the photograph, i.e., at 12 o'clock.
- 10 µl of total SRFs released during 12 hr in pH neutral buffer by 10^9 colony-forming-units of *L. monocytogenes* per ml. were applied at site "1."
- 10 µl of SRFs <10 kDa released during 12 hr in pH neutral buffer by 10^9 colony-forming-units of *L. monocytogenes* per ml. were applied at site "2."
- 10 µl of SRFs <10 kDa released during 12 hr in pH 4 buffer by 10^9 colony-forming-units of a mixture of five strains of *L. plantarum* and two strains of *E. faecium* per ml. were applied at site "3."

RESULTS & CONCLUSIONS

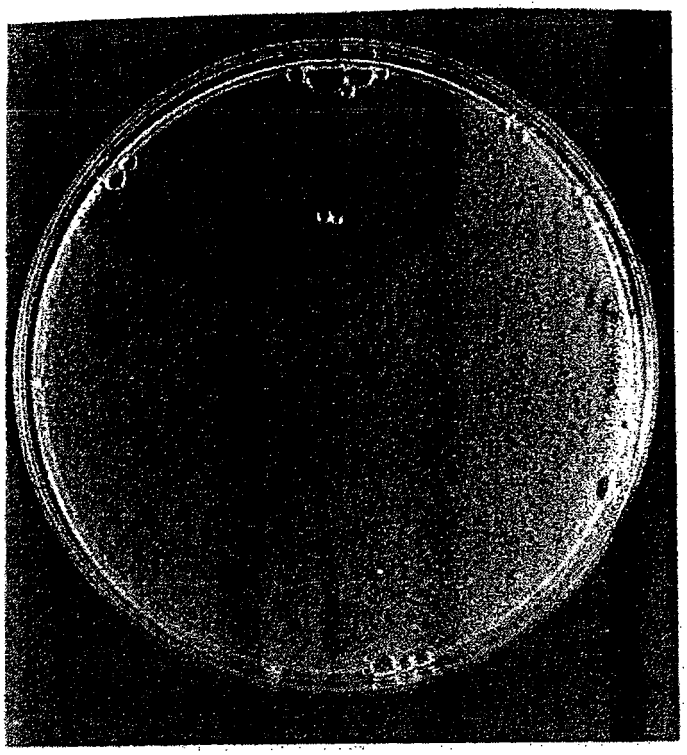
The clear area around the application point of Nisin demonstrates the inhibitory powers of bacteriocins.

No clear zones formed around the spots where SRFs were added indicate that SRFs do not possess bacteriocin or bacteriocin-like activity.

FIGURE 2

SRI Do t Possess Bacteriocin-like tiv

L. monocytogenes, L. plantarum and E. faecium do not release bacteriocins by exposure to pH neutral buffer or pH 4 buffer.



PROCEDURE:

A lawn of *Lactobacillus helveticus* ATCC 15009 was prepared by pour plating the test strain used by De Vuyst for the detection of bacteriocin.

Three hr later:

- 10 µl containing 25 µg of Nisin, a known bacteriocin, from Sigma-Aldrich was added to the solidified agar at the top spot in the photograph, i.e., at 12 o'clock.
- 10 µl of SRFs <10 kDa released during 12 hr in pH neutral buffer by 10^{10} colony-forming-units of *L. monocytogenes* per ml. were applied at site "4."
- 10 µl of SRFs <10 kDa released by 10^{9} colony-forming-units of a mixture of five strains of *L. plantarum* and two strains of *E. faecium* per ml. first exposed to pH 4 for 3 hr, then pH 7 for 12 hr were applied at site "5."
- 10 µl of SRFs <10 kDa released by 10^{9} colony-forming-units of a heat killed mixture of five strains of *L. plantarum* and two strains of *E. faecium* per ml. first exposed to pH 4 for 3 hr, then pH 7 for 12 hr were applied at site "6."

RESULTS & CONCLUSIONS

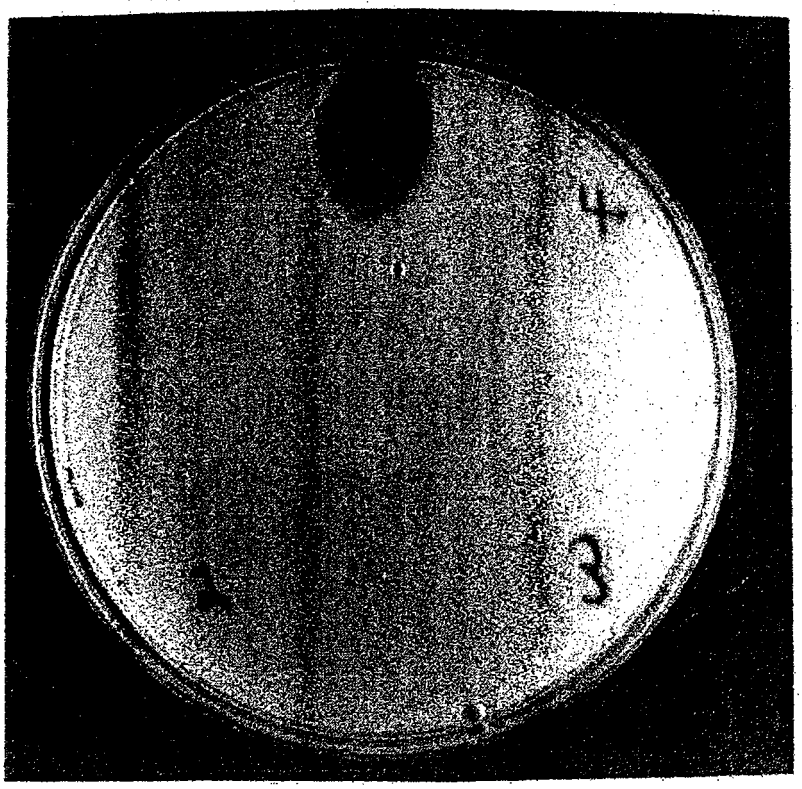
The clear area around the application point of Nisin demonstrates the ability of the bacteriocin to inhibit the growth of bacteria.

No clear zones formed around the spots where SRFs were added indicate that SRFs do not possess bacteriocin or bacteriocin-like activity.

FIGURE 3

SRFs Do Not Possess Bacteriocin-like Activity

L. caseii, L. plantarum or *E. faecium* do not release bacteriocins during exposure to pH neutral buffer or pH 4 buffer.



PROCEDURE:

A lawn of *Lactobacillus helveticus* ATCC 15009 was prepared by pour plating the test strain used by De Vuyst for the detection of bacteriocin.

Three hr later:

- 10 µl containing 25 µg of Nisin, a known bacteriocin from Sigma-Aldrich was added to the solidified agar at the top spot in the photograph, i.e., at 12 o'clock.
- 10 µl of total SRFs released by 10^9 colony-forming-units of *L. caseii* per ml during 12 hr in pH neutral buffer were applied at site "1."
- 10 µl of SRFs <10 kDa released by 10^9 colony-forming-units of *L. caseii* per ml during 12 hr in pH neutral buffer were applied at site "2."
- 10 µl of total SRFs released by 10^9 colony-forming-units of a mixture of 5 strains of *L. plantarum* per ml and 2 strains of *E. faecium* during 12 hr in pH 7 buffer were applied at site "3."
- 10 µl of SRFs <10 kDa released by exposing 10^9 colony-forming-units of a mixture of 5 strains of *L. plantarum* per ml and 2 strains of *E. faecium* during 12 hr in pH 7 buffer were applied at site "4."

RESULTS & CONCLUSIONS

The clear area around the application point of Nisin demonstrates the ability of bacteriocin to inhibit the growth of bacteria.

No clear zones formed around the spots where SRFs were added indicate that SRFs do not possess bacteriocin or bacteriocin-like activity.

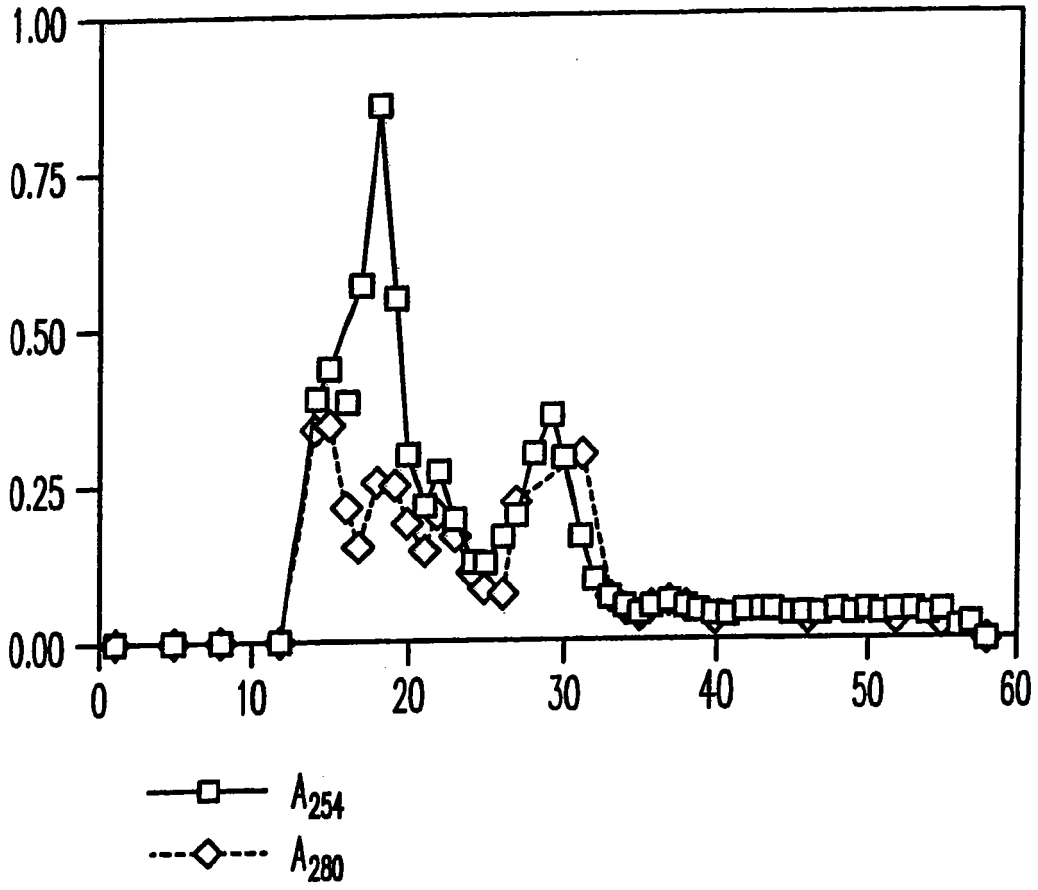


Figure 4

Shows the <10 kDa SRFs released by a direct-fed-microbial or animal probiotic commercially marketed which indicates the presence of macrophage-activating oligomers.

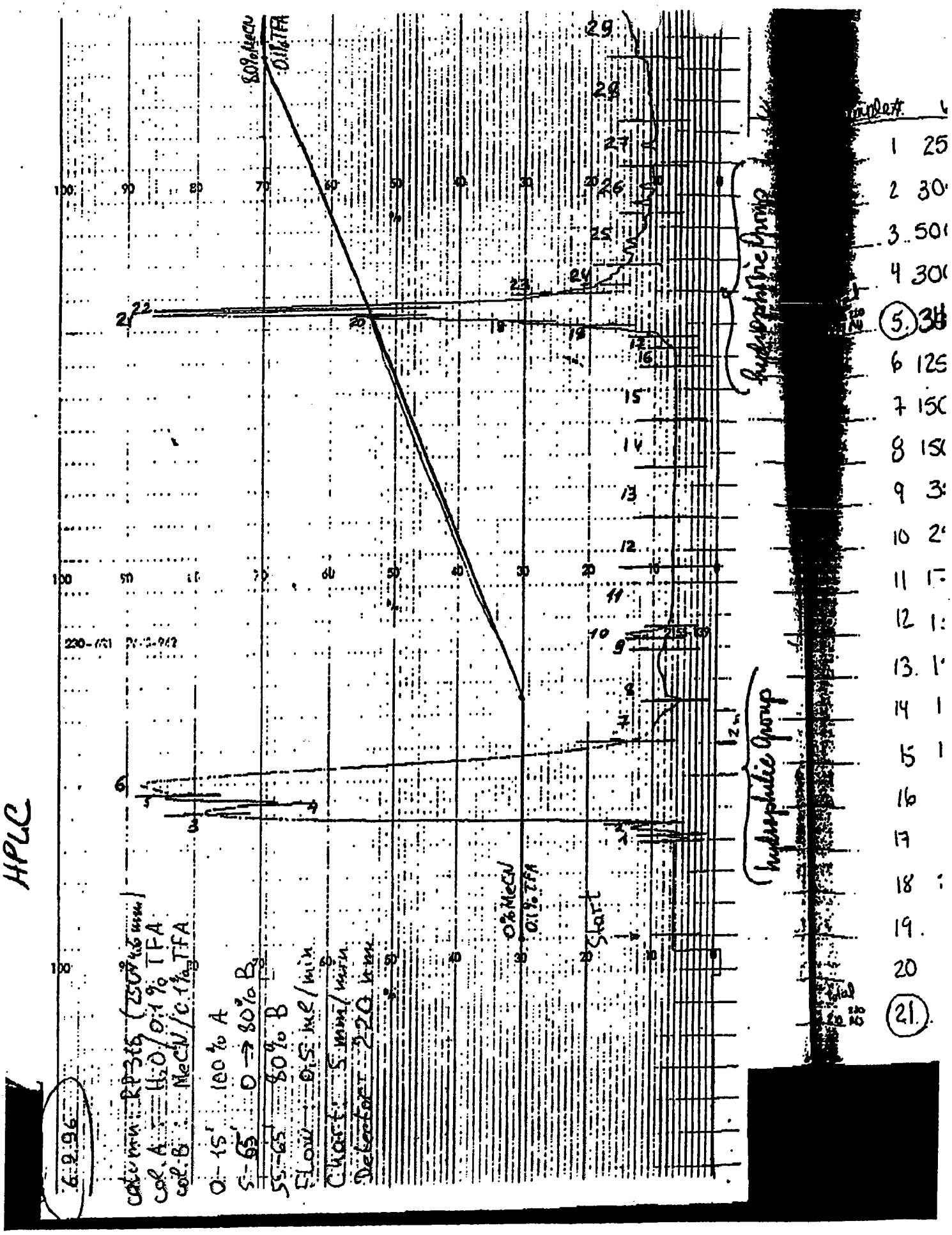

HPLC
6.2.96
Column: RP3t5 (250 x 4.6 mm)
Col. A = H2O/0.1% TFA
Col. B = MeCN/c 1% TFA
0-15' 100% A
5-65 0 → 80% B
55-65 80% B
Flow 0.5 ml/min
Chart 5 mm/min
Detector 220 nm
50% MeCN
0.1% TFA
0% MeCN
0.1% TFA
Start
hydrophilic group
hydrophobic group
Inject #
1 25
2 30
3 50
4 30
5. 3
6 12
7 15
8 15
9 3
10 2
11 1
12 1
13. 1
14 1
15 1
16
17
18
19.
20
21.

FIGURE 5

FIGURE 6
SRFs <10 kDa from *L. monocytogenes* Are Oligoribonucleotides

PROCEDURE:

Glass plates coated with silica were purchased from E-M Science, Dusseldorf, Germany. Solvent was n-Propanol:ammonium hydroxide:water, 6:3:1, by volume according to Grippo, P., M. Iaccarino, M.Rossi, and E. Scarano. 1965. Thin-layer chromatography of nucleotides, nucleosides and nucleic acid bases. Biochim. Biophys. Acta 65:95: 1-7.

Aliquots of 10 μl of the following were spotted in the silica on a line 2.5 cm from the bottom of the plate. They were allowed to dry for an hour at room temperature before the plate was put into a closed chamber containing the solvent, about 0.5 cm deep. The solvent was allowed to develop for 3½hr up the plate for 13.5 cm at room temperature. The plate was removed from the chamber, dried at room temperature and photographed under an ultraviolet light of absorbance 254 nm.

Spotted in lanes:

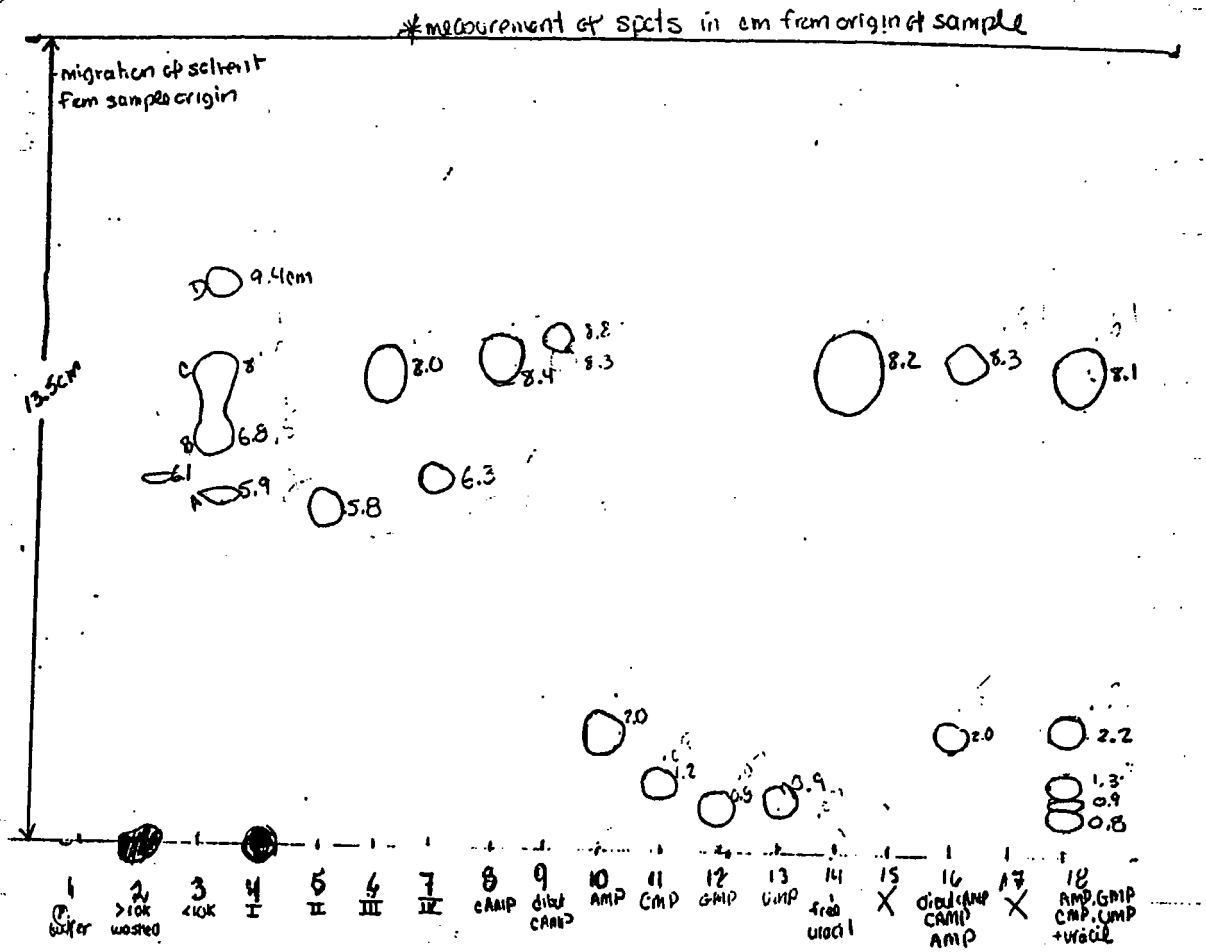
1. pH neutral buffer
2. SRFs >10kDa released by 10^9 colony-forming-units of *L. monocytogenes* for 12 hr in pH neutral buffer.
3. SRFs <10kDa released by 10^9 colony-forming-units of *L. monocytogenes* for 12 hr in pH neutral buffer.
4. Sephadex Peak I of SRFs <10kDa released by 10^9 colony-forming-units of *L. monocytogenes* for 12 hr in pH neutral buffer.
5. Sephadex Peak II SRFs <10kDa released by 10^9 colony-forming-units of *L. monocytogenes* for 12 hr in pH neutral buffer.
6. Sephadex Peak III SRFs <10kDa released by 10^9 colony-forming-units of *L. monocytogenes* for 12 hr in pH neutral buffer.
7. Sephadex Peak IV SRFs <10kDa released by 10^9 colony-forming-units of *L. monocytogenes* for 12 hr in pH neutral buffer.
8. Cyclic AMP standard
9. dibutyl cyclic AMP standard
10. AMP
11. CMP – cytidine monophosphate standard
12. GMP – guanosine monophosphate standard
13. UMP – uridine monophosphate standard
14. free uracil
15. vacant
16. combination of dibutyl cyclic AMP, Cyclic AMP and AMP
17. vacant
18. combination of AMP, GMP, CMP and uracil

RESULTS & CONCLUSIONS:

L. monocytogenes releases ribonucleotides during a 12-hr exposure to pH neutral buffer. The fraction <10kDa contains oligoribonucleotides.

Silica plate ~ 1 yr

Results:



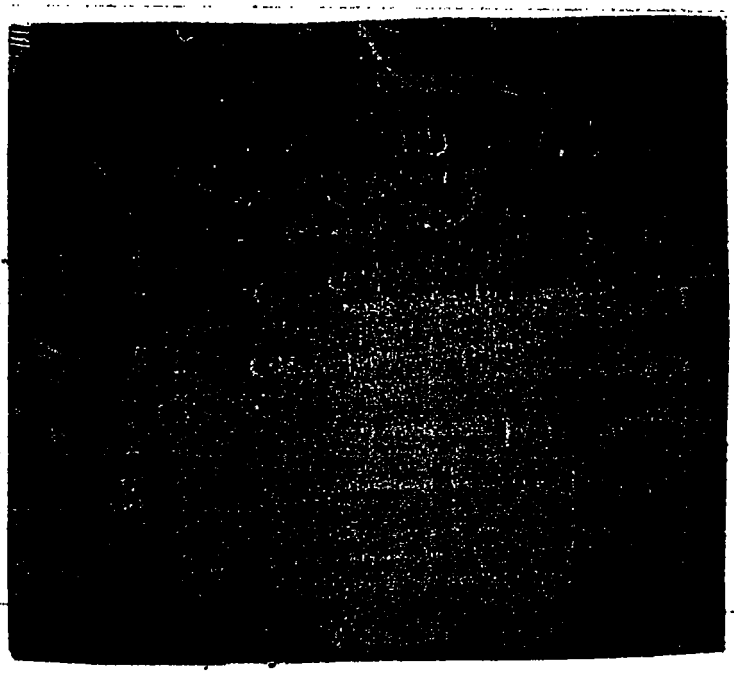


FIGURE 7

SRFs <10 kDa from *L. caseii* Are Oligoribonucleotides, Similar to those Released by *L. monocytogenes*.

PROCEDURE:

Glass plates coated with silica were purchased from Boehringer-Mannheim. Solvent was n-Propanol:ammonium hydroxide:water, 6:3:1, by volume according to Grippo, P., M. Iaccarino, M.Rossi, and E. Scarano. 1965. Thin-layer chromatography of nucleotides, nucleosides and nucleic acid bases. Biochim. Biophys. Acta 65:95: 1-7.

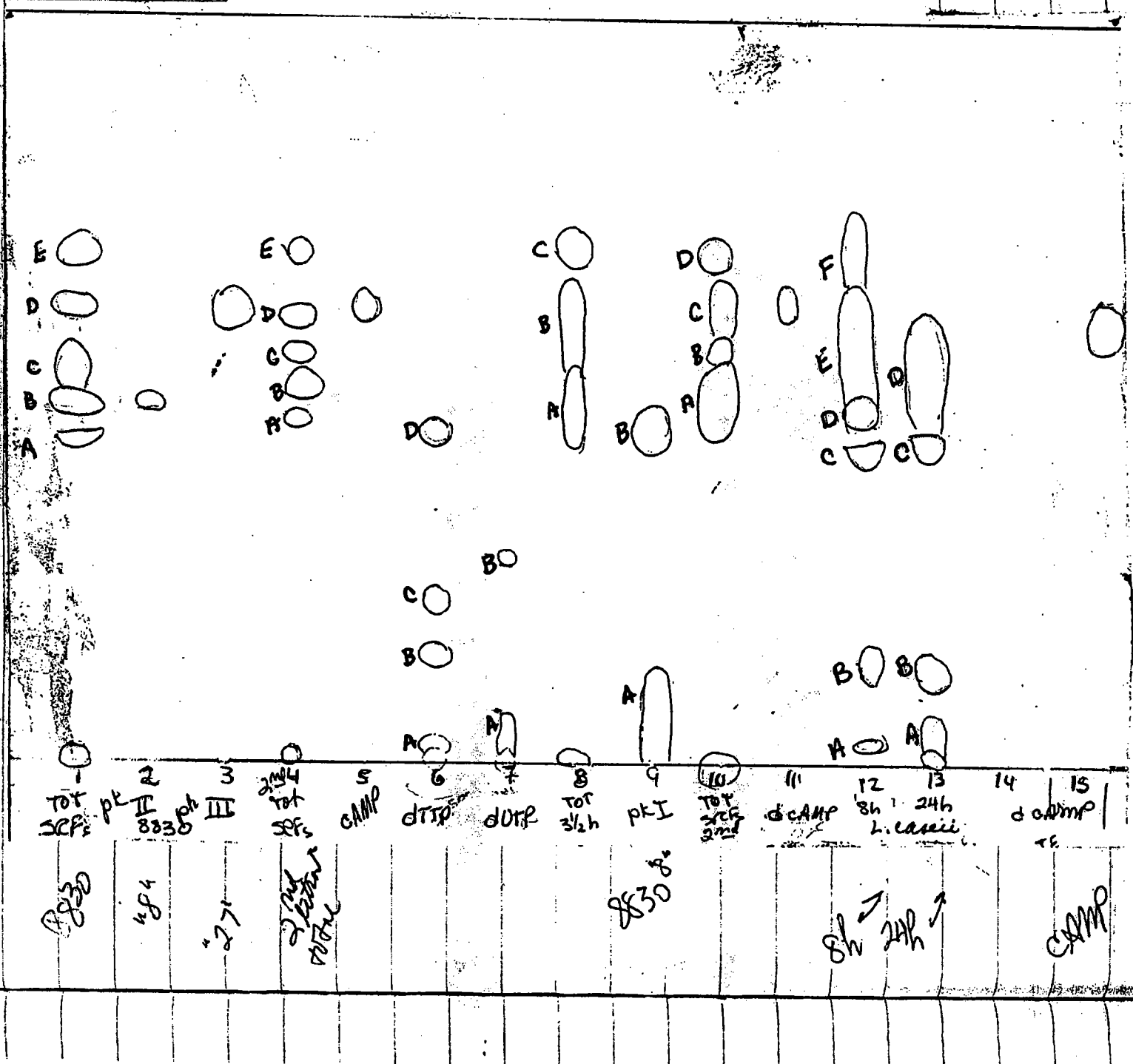
Aliquots of 10 μl of the following were spotted in the silica on a line 2.5 cm from the bottom of the plate. They were allowed to dry for an hour at room temperature before the plate was put into a closed chamber containing the solvent, about 0.5 cm deep. The solvent was allowed to develop for 3½hr up the plate for 13.5 cm at room temperature. The plate was removed from the chamber, dried at room temperature and photographed under an ultraviolet light of absorbance 254 nm.

Spotted in Lanes:

1. Total SRFs released by 10^9 colony-forming-units of *L. monocytogenes* for 12 hr in pH neutral buffer.
2. Sephadex Peak II SRFs <10kDa released by 10^9 colony-forming-units of *L. monocytogenes* for 12 hr in pH neutral buffer.
3. Sephadex Peak III SRFs <10kDa released by 10^9 colony-forming-units of *L. monocytogenes* for 12 hr in pH neutral buffer.
4. Total SRFs released by 10^9 colony-forming-units of *L. monocytogenes* during 3½ hr in pH neutral buffer after an initial 12 hr exposure in pH neutral buffer.
5. Cyclic AMP standard
6. dibutyl thymidine triphosphate standard
7. dibutyl uridine triphosphate
8. Same as 4.Total SRFs released by 10^9 colony-forming-units of *L. monocytogenes* during 3½ hr in pH neutral buffer after an initial 12 hr exposure in pH neutral buffer.
9. Sephadex Peak I of SRFs <10kDa released by 10^9 colony-forming-units of *L. monocytogenes* for 12 hr in pH neutral buffer.
10. Same as 4.
11. dibutyl cyclic AMP
12. Total SRFs released by 10^9 colony-forming-units of *L. caseii* during 8 hr in pH neutral buffer.
13. Total SRFs released by 10^9 colony-forming-units of *L. caseii* during 24
14. Vacant.
15. dibutyl cyclic AMP

RESULTS & CONCLUSIONS:

Both *L. monocytogenes* and *L. caseii* release ribonucleotides during exposures to pH neutral buffer. Their oligoribonucleotides <10kDa are similar.


1 TOT SCFs
2 pk II 8830 pk
3 III
4 2nd TOT SCFs
5 cAMP
6 dTTP
7 dUTP
8 TOT 3½h
9 pk I
10 TOT SCFs 2nd
11 dcAMP
12 8h L. casei
13 24h
14
15 dcAMP
TE
8830
484
727
8h
24h
cAMP

0.04

0.13

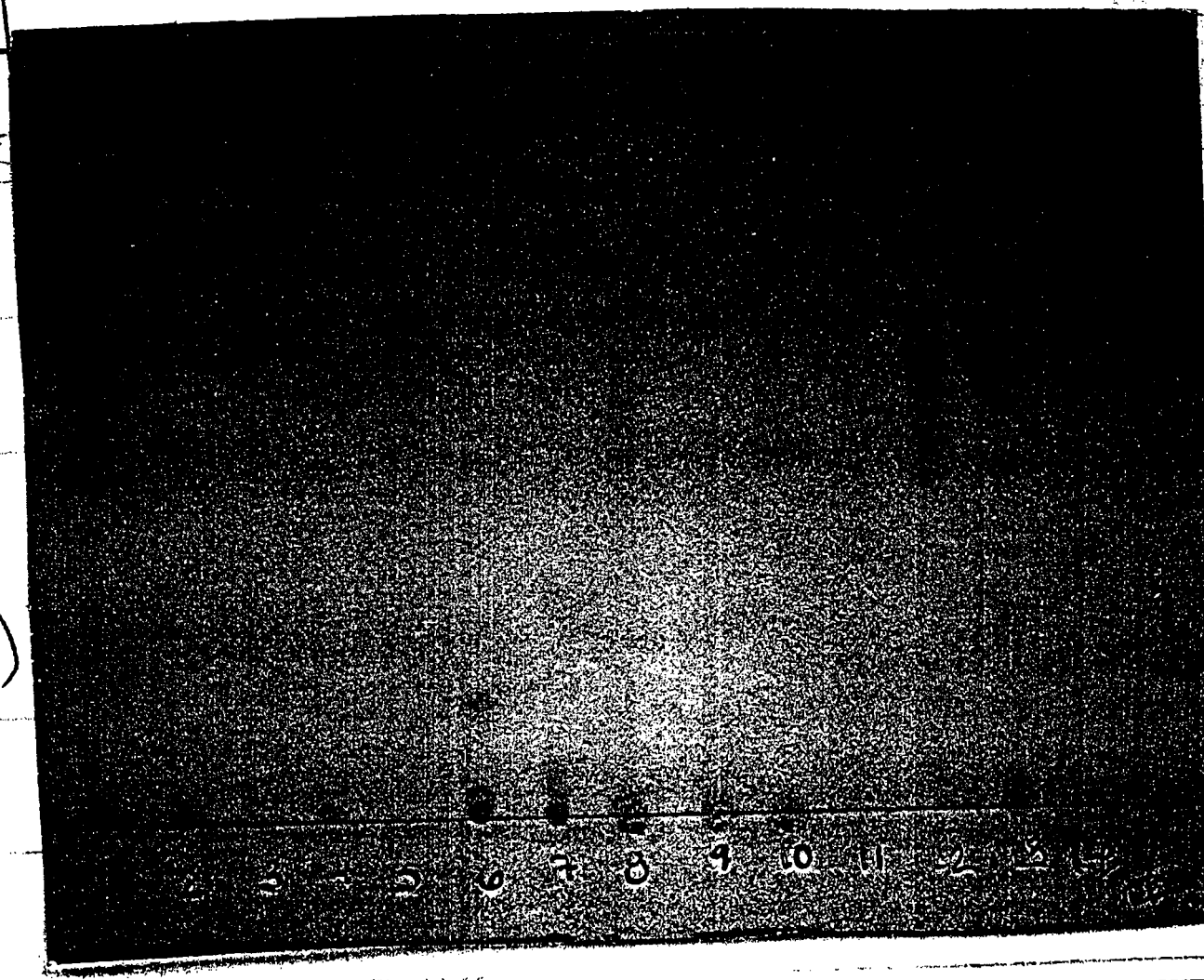
0.43

0.54

0.59 *

Similar to breakdown product of dTTP (maybe dTMP or dTDP?)

8830 —— * —— 393



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ter UV 11.
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of dots on TLC plate)

X. RELATED PROCEEDINGS APPENDIX

There are no related proceedings in the present appeal of U.S. Application No. 09/883,550.

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